UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

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Chemical: Difenoconazole
PC Code: 128847
DP Barcodes: D344681

MEMORANDUM

SUBJECT: Syngenta Response to comments to the Environmental Fate and Effects Division Risk Assessment for the Section 3 New Use Registration of Difenoconazole

TO: Janet Whitehurst, Chemical Review Manager
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FROM: Holly Galavotti, Environmental Protection Specialist
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Holly Galavotti 10/24
10/24/07

Please find the attached Environmental Fate and Effects Division's (EFED) response to Syngenta's comments regarding the environmental risk assessment for the proposed new use registration of the fungicide, difenoconazole. The proposed-label evaluated in the risk assessment is Inspire® (100-XXXX) for use on fruiting vegetables, pome fruit, vegetables subgroup (tuberous and corm), sugar beets, and ornamentals.

Syngenta requested further consideration of the results of the difenoconazole mysid chronic toxicity study (MRID 469501-33). EFED has reviewed these comments and concluded that this study will remain classified as Supplemental with reproductive success (NOAEC < 0.155 µg/L) being the most sensitive endpoint. In the study, reproductive success was reduced at all treatment levels compared to the negative control (36-56 % reduction). This endpoint was used in the risk assessment and indicates potential significant adverse effects to estuarine/marine invertebrates exposed to difenoconazole. Risk quotients (RQs) for chronic estuarine/marine crustacean toxicity are based on this non-definitive endpoint. RQs range from >11.22 to > 99.13 for all the

proposed uses.

Syngenta requested further consideration of the application of the EPA memo entitled, "Interim Policy Guidance for the Use of Dilution-Water (Negative) and Solvent Controls in Statistical Data Analysis for Guideline Aquatic Toxicology Studies", dated March 30, 2006, to this mysid study. This memo states that the treatment groups should be compared to the negative control when no solvent effects exist. In this mysid study, there is a 37% reduction in reproduction in the solvent control (3.4 young/female) compared to the negative control (5.4 young/female). Although this is not statistically significant, there is a question of its biological significance. Because there were only two replicates for each treatment, identifying differences between the two controls was difficult and the test may not identify differences as significant even if they exist. In addition, there is a slight (3%), but statistically significant reduction in female body length of solvent control mysids. Therefore, female body length was not included as an endpoint for the risk assessment. It is a standard principle in the conduct of toxicity tests that a solvent, if used, should not affect the toxicity of the test material or the organism (ASTM E729) and this is clearly stated in EPA guidance (*e.g.* OPPTS Guideline 850.1350). The review compared the treatments to the negative control to determine NOAEC levels in accordance to the best professional judgment that the solvent should not impact the treatment groups.

This study was also classified as supplemental because there was relatively high test material variability observed at all test concentrations (except the nominal 3.00 µg ai/L treatment), with measured concentrations differing by more than 20% of the nominal (24-43% difference). In addition, the stability of exposure concentration could not be established because it is unclear the number of times the stock solution was replaced and whether or not the stock solution was measured after the initiation of the test. It should be indicated when the treatment concentrations were measured relative to the time the stock solution was replaced. Each endpoint of the study is discussed below.

Percent Survival

There were no significant differences between the negative control and the solvent control for female, male, or combined sex survival at the termination of the 28-day test. There were no statistically significant treatment effects for female, male, or combined survival compared the negative control; therefore, the NOAEC = 8.14 µg ai/L and the LOAEC > 8.14 µg ai/L. The following table indicates the statistical methods used and the NOAEC and LOAEC values for each endpoint combination.

Endpoint	Method	NOAEC	LOAEC
Male Survival	Dunnett's test (no dose response)	8.14 µg ai/L	>8.14 µg ai/L
Female Survival	Kruskal-Wallis with Dunn's multiple comparison (failed homogeneity of variance test, no dose response)	8.14 µg ai/L	>8.14 µg ai/L
Combined Sex Survival	Dunnett's test (no dose response)	8.14 µg ai/L	>8.14 µg ai/L

Reproductive Success

Reproductive success was determined by evaluating the number of offspring/female and the average number of offspring/ female/ reproductive day. The study author calculated the number of offspring/ female/ reproductive day as the ratio of the number of young released to the number of days that an individual female was alive, counting from the day the offspring were first observed in the control (usually 13 days). However, by this method, females that had produced young and then died before the test was finished were assigned a higher reproductive rate than females that had lived to the end of the test. Therefore, the number of offspring/ female should be divided by 13 reproductive days for all individuals.

Based on the t-test, there were no significant differences between the negative control and the solvent control at an alpha value of 0.05. Based on the William's test, there are significant differences at all treatment levels for the number of young per female (NOAEC <0.115 µg ai/L) compared to the negative control. The results are the same for the number of young/female/reproductive day because the number of reproductive days is the same for all samples (13 days). The William's test is generally based on a monotonic decrease. The reduction of the number of young produced per female is considered to generally show a monotonic trend when you consider variability in the trend. There was 36%, 47%, 45%, 56%, and 53% decrease for each treatment compared to the negative control. Based on the Dunnett's test, which does not require a monotonic decrease, there are significant differences at the 2.58 µg ai/L treatment level (NOAEC = 0.774 µg ai/L). Because there are only two replicates, these tests have little power. The Dunnett's test is only able to detect differences of 50.6% and greater. Due to these uncertainties regarding statistical power, the conservative result of the Williams Test are used as the endpoint for risk assessment (NOAEC < 0.115 µg ai/L).

Endpoint	Method	NOAEC	LOAEC
Number of young/ female and number of young/female/day	Dunnett's test (compared to the negative control)	0.786 µg ai/L	2.60 µg ai/L
	William's Test (compared to the negative control)	<0.115 µg ai/L	0.115 µg ai/L

When the treatments are compared to the solvent control, the William's and the Dunnett's tests show that there are no significant differences at any treatment level for the number of offspring/female. The Dunnett's test is only able to detect differences of 56.1% and greater using the solvent control.

It should be reiterated that because there are only two replicates in this study, these tests have little power to detect differences from the control groups as demonstrated by only being able to detect a greater than 50% difference in the Dunnett's Test. Although there are no statistical differences between the negative and solvent control groups, there is a 37% reduction in reproduction in the solvent control indicating that a solvent effect may exist. Based on these uncertainties, this study is deemed supplementary information and the conservative approach is used for risk assessment purposes in which the NOAEC < 0.115 µg ai/L.

Growth

The NOAEC value for growth based on male dry weight is 0.311 µg ai/L. The NOAEC value for female dry weight is 2.6 µg ai/L. There is a slight (3%), but statistically significant reduction in female body length of solvent control mysids, when compared to the negative control mysids. Female body length was also slightly lower (3-5%), but statistically significant, than the negative control at all but the lowest treatment level. While the biological significance of a 3-5% reduction in female body length may be minimal, the significant difference between solvent and negative control mysid lengths suggests that adverse effects may not have been due to the active ingredient alone.

There was no significant difference between males and females for total length based on a t-test. Therefore, the total length results were combined for males and females. The combined results also showed a significant difference between the negative and the solvent control. Therefore female length was not included as an endpoint in the results of this study. There was not a significant difference between the negative and solvent control for male length and the NOAEC was determined to be 8.14 µg ai/L.

Endpoint	Method	NOAEC	LOAEC
Total Length Male	Kruskal-Wallis with Dunn's multiple comparison	8.14 µg ai/L	>8.14 µg ai/L
Total Length Female	Significant difference between negative and solvent control	ND	ND
Combined Total Length	Significant difference between negative and solvent control	ND	ND
Dry Weight Male	William's test (exhibits dose response)	0.311 µg ai/L	0.786 µg ai/L
Dry Weight Female	William's test (exhibits dose response)	2.60 µg ai/L	8.14 µg ai/L

ND = Not Determined because there were significant differences between the negative and solvent control.

Statistical Output

Number of Young/ Female

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t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	5.4000	CALCULATED t VALUE =	1.4359
GRP2 (BLANK CRTL) MEAN =	3.4000	DEGREES OF FREEDOM =	2
DIFFERENCE IN MEANS =	2.0000		

TABLE t VALUE (0.05 (2), 2) = 4.303 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 2) = 9.925 NO significant difference at alpha=0.01

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Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	0.804	2.904	4.584	2.904	0.804
OBSERVED	0	6	0	6	0

Calculated Chi-Square goodness of fit test statistic = 12.7934
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

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Shapiro Wilks test for normality

D = 5.605

W = 0.991

Critical W (P = 0.05) (n = 12) = 0.859
Critical W (P = 0.01) (n = 12) = 0.805

Data PASS normality test at P=0.01 level. Continue analysis.

File: offsprin.txt Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 6.47
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 1.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	12.424	2.485	2.661
Within (Error)	6	5.605	0.934	
Total	11	18.029		

Critical F value = 4.39 (0.05,5,6)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

File: offspring.txt Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	5.400	5.400		
2	0.115	3.450	3.450	2.018	
3	0.311	2.850	2.850	2.639	
4	0.786	2.950	2.950	2.535	
5	2.60	2.350	2.350	3.156	*
6	8.14	2.550	2.550	2.949	*

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

File: offspring.txt Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	2			
2	0.115	2	2.735	50.6	1.950
3	0.311	2	2.735	50.6	2.550
4	0.786	2	2.735	50.6	2.450
5	2.60	2	2.735	50.6	3.050
6	8.14	2	2.735	50.6	2.850

File: offspring.txt Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	5.400	5.400	5.400
2	0.115	2	3.450	3.450	3.450
3	0.311	2	2.850	2.850	2.900
4	0.786	2	2.950	2.950	2.900
5	2.60	2	2.350	2.350	2.450
6	8.14	2	2.550	2.550	2.450

File: offspring.txt Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	5.400				
0.115	3.450	2.018	*	1.94	k= 1, v= 6
0.311	2.900	2.587	*	2.06	k= 2, v= 6

0.786	2.900	2.587	*	2.10	k= 3, v= 6
2.60	2.450	3.052	*	2.12	k= 4, v= 6
8.14	2.450	3.052	*	2.13	k= 5, v= 6

s = 0.967

Note: df used for table values are approximate when v > 20.

treatment compared to the solvent control
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1.957	0.391	0.861
Within (Error)	6	2.725	0.454	
Total	11	4.682		

Critical F value = 4.39 (0.05,5,6)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

treatment compared to the solvent control
File: off2.txt Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	3.400	3.400		
2	0.115	3.450	3.450	-0.074	
3	0.311	2.850	2.850	0.816	
4	0.786	2.950	2.950	0.668	
5	2.60	2.350	2.350	1.558	
6	8.14	2.550	2.550	1.262	

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

treatment compared to the solvent control
File: off2.txt Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	2			
2	0.115	2	1.907	56.1	-0.050
3	0.311	2	1.907	56.1	0.550
4	0.786	2	1.907	56.1	0.450
5	2.60	2	1.907	56.1	1.050
6	8.14	2	1.907	56.1	0.850

treatment compared to the solvent control
File: off2.txt Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	3.400	3.400	3.425
2	0.115	2	3.450	3.450	3.425
3	0.311	2	2.850	2.850	2.900
4	0.786	2	2.950	2.950	2.900
5	2.60	2	2.350	2.350	2.450
6	8.14	2	2.550	2.550	2.450

treatment compared to the solvent control
File: off2.txt Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	3.425				
0.115	3.425	0.037		1.94	k= 1, v= 6
0.311	2.900	0.742		2.06	k= 2, v= 6
0.786	2.900	0.742		2.10	k= 3, v= 6
2.60	2.450	1.410		2.12	k= 4, v= 6
8.14	2.450	1.410		2.13	k= 5, v= 6

s = 0.674

Note: df used for table values are approximate when v > 20.